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Letter to the Editor
Receptors for Hepatitis C Virus

The nature of the receptors enabling hepatitis C virus (HCV) entry into cells has been the subject of debate. On one hand, ligand panning with recombinant HCV envelope protein has identified CD81, a member of the tetraspanin superfamily of proteins, as a direct ligand for the E2 glycoprotein (7) but fails to explain virus tropism. On the other, virus present in infected blood is found associated with low-density lipoprotein (LDL) and the LDL receptor (LDL-R) has been suggested as a receptor for the virus (1, 6). Although the use of LDL-R would explain tropism, there is no evidence for the viral glycoprotein binding directly to the receptor. Equally, direct comparison of the reports identifying proposed viral ligands is made difficult by the fact that one set of data uses virus as the source of E protein while the other uses isolated E2. Here, using purified recombinant E1-E2 complex and an alternate ligand selection procedure, we identified a single peptide with tight binding to E2 and homology with the LDL-R.

HCV, a member of the *Flaviviridae*, is the causative agent of the majority of cases of non-A, non-B hepatitis. The virus encodes two glycoproteins, E1 and E2, which are produced initially as a part of the single virion precursor protein but later mature by proteolytic cleavage to form a noncovalently linked heterodimer. The level of antibody response to E2 correlates with protection in animal models (4) and with occasional clearance of virus in natural infections (5), suggesting that it is the major receptor binding protein of the virus. Although purified recombinant E2 clearly binds to CD81 with high affinity (7), a role for the E1-E2 complex as found on the virion, and in which the E2 conformation may be influenced by the presence of E1, cannot be ruled out. We therefore expressed recombinant E1-E2 fused to the carrier protein glutathione-*S*-transferase in eukaryotic cells (9). During expression, the E1-E2 junction is cleaved and an E1-E2 complex can be purified from cell lysates by chromatography on immobilized glutathione. The complex has been characterized and binds to CD81 (3). Purified E1-E2 complex was immobilized onto polystyrene and used as substrate for panning a phage peptide display library of random unconstrained 15-mer peptides (8). Unbound phage were removed, and those remaining were recovered, titrated, and regrown for further rounds of panning. The titer of adherent phage plateaued after four rounds of panning, whereupon 19 individual phages were isolated, their relative binding to the E1-E2 complex was determined by enzyme-linked immunosorbent assay (ELISA), and the encoded peptides were determined by nucleotide sequencing. Eighty-four percent of the isolates (16 of 19) had one peptide sequence and bound isolated E1-E2 protein with the highest affinity (Fig. 1). The remainder had various sequences and lesser binding affinities.

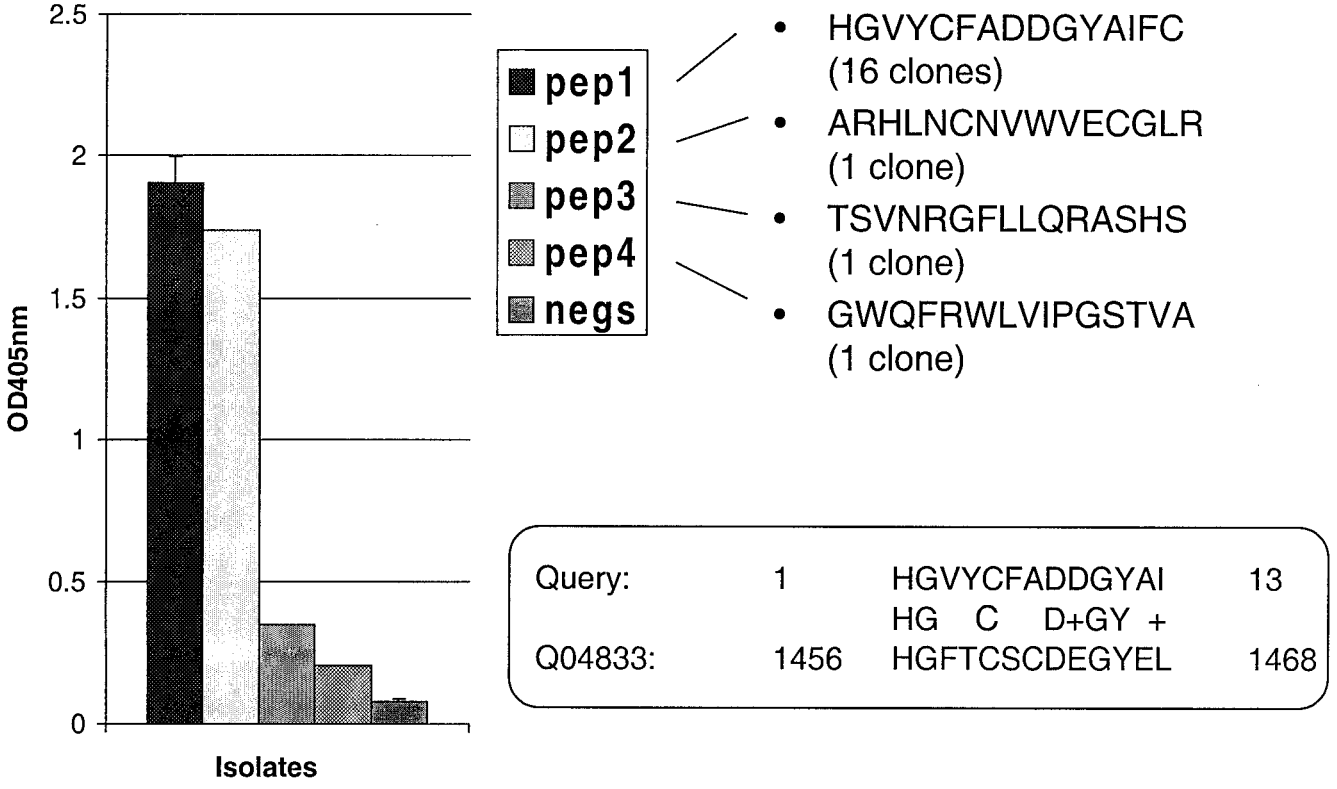

FIG. 1. (Left) Phage isolate binding to immobilized E1-E2 by ELISA, using anti-phage coat protein antibody. (Upper right) Sequence of 19 isolated peptides that bind to E1-E2. (Lower right) Alignment of the predominant peptide with Q04833.

Searches of the databases identified a match between the predominant peptide and an LDL-R-related molecule from *Caenorhabditis elegans* (Q04833) (Fig. 1) (lower right). A related sequence is present in the human LDL-R, but the homology is less, suggesting that a related molecule may be the authentic ligand.

Notwithstanding E2 binding with CD81, our finding that recombinant HCV E protein can bind to an LDL-R-like sequence is consistent with an LDL-R-like molecule acting as a receptor for HCV virion endocytosis (1), as it does for some other viruses (6).

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